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     (FILE 'HOME' ENTERED AT 11:04:30 ON 21 DEC 2006)
     FILE 'CA' ENTERED AT 11:04:38 ON 21 DEC 2006
L1 207053 S (KINASE OR (COVALENT? OR CHEMICAL?)(2A)MODIF? OR PHOSPHORYLA?)
          (7A) (PEPTIDE OR PROTEIN OR SUBSTRATE OR HCG OR CHORIONIC
          GONADOTROPHIN)
L2   7001 S L1 AND (FLUORESC? OR LUMINESC?)
L3  79443 S (BILAB? OR DILAB? OR MULTILAB? OR (DUAL OR BI OR TWO OR 2 OR
          PLURAL? OR MULTI? OR DOUBLE)(3A)(DYE OR LABEL? OR TAG OR INDICATOR
          OR FLUOROPHORE OR LUMINOPHORE OR CHROMOPHORE))
L4  27127 S (DYE OR LABEL? OR TAG OR INDICATOR OR NON(1A)(LUMINESC? OR
          FLUORESC?) OR NONLUMINESC? OR NONFLUORESC?)(5A)(DIMER OR STACK? OR
          AGGREGAT? OR COMPLEX?)
L5    336 S L2 AND L3
L6     50 S L2 AND L4
L7    361 S L2 AND QUENCH?
L8     22 S L5-6 AND L7
L9      7 S L2 AND(HCG OR GONADOTROPHIN)
L10    69 S L3 AND(HCG OR GONADOTROPHIN)
L11    44 S L4 AND(HCG OR GONADOTROPHIN)
L12   129 S L6,L8-11 AND PY<2000
     FILE 'BIOSIS' ENTERED AT 11:14:36 ON 21 DEC 2006
L13    97 S L12
     FILE 'MEDLINE' ENTERED AT 11:15:20 ON 21 DEC 2006
L14    92 S L12
     FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 11:16:44 ON 21 DEC 2006
L15   196 DUP REM L8 L12 L13 L14 (144 DUPLICATES REMOVED)

=> d bib,ab l15 1-196

L15  ANSWER 3 OF 196  CA  COPYRIGHT 2006 ACS on STN
AN   142:193980  CA
TI   Use of chimeric fluorescent proteins as reagents to determine kinase and
     phosphatase activity
IN   Violin, Jonathan D.; Newton, Alexandra C.; Tsien, Roger Y.; Zhang, Jin
PA   USA
SO   U.S. Pat. Appl. Publ., 68 pp., Cont.-in-part of U.S. Ser. No. 865,291.
PI   US 2005026234        A1    20050203    US 2004-857622          20040528
     US 5981200           A     19991109    US 1997-792553          19970131
PRAI US 1996-594575       A2    19960131
AB   The present invention relates generally to reagents for detg. kinase and
     phosphatase activity, and more specifically to chimeric proteins contg.
     two fluorescent proteins and a phosphorylatable domain, and methods of
     using such chimeric proteins to detect kinase or phosphatase activity.
     The present invention provides compns. and methods that are generally
     useful for nondestructively detecting and monitoring protein kinase and
     phosphatase activities in individual living eukaryotic cells, including
     mammalian cells, and provide a means to obtain spatial and temporal
     resoln. on the order of a few micrometers and seconds, or better.  As
     disclosed herein, protein kinase and phosphatase activities can be
     monitored using chimeric substrates (chimeric phosphorylation
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indicators) that incorporate reporter mols. such as **fluorescent** proteins or **luminescent** complexes, whose properties change significantly as a function of the **phosphorylation** state of the **substrate**. Protein interactions are detected by resonance energy transfer using **fluorescent** proteins or lanthanide **complexes** to **label** the putative partners. The compns. of the invention are adaptable to modification using methods such as high throughput combinatorial generation and screening techniques and, therefore, readily can be varied to allow monitoring of any desired **kinase**, phosphatase, or **protein** interaction. Accordingly, the present invention provides chimeric phosphorylation indicators having various structures as disclosed herein. In a first embodiment, a chimeric phosphorylation indicator contains, in operative linkage, a donor mol., a phosphorylatable domain, a phosphoaminoacid binding domain, and an acceptor mol., wherein the phosphoaminoacid binding domain specifically binds to a phosphoaminoacid when present in the phosphorylatable domain, the donor mol. and the acceptor mol. exhibit a detectable resonance energy transfer when the donor is excited, and the phosphorylatable domain and phosphoaminoacid binding domain do not substantially emit light to excite the acceptor. The authors generated genetically-encoded **fluorescent** reporters for PKC activity that reversibly respond to stimuli activating PKC. Specifically, phosphorylation of the reporter expressed in mammalian cells causes changes in **fluorescence** resonance energy transfer (FRET), allowing real time imaging of phosphorylation resulting from PKC activation. A reporter for PKC-mediated phosphorylation (C Kinase Activity Reporter, CKAR) was constructed analogously to a previously described reporter (AKAR) for **protein kinase** A (Zhang et al., 2001). CKAR was successfully designed to be a specific, reversible reporter of phosphorylation by PKC (FIGS. 8 and 9). The foregoing results demonstrate that a genetically engineered reporter may be used for monitoring the activity of endogenous PKC. These indicators may have significant value in flow cytometry, complex tissues, or high-throughput screening, where quant. monitoring of translocation of a GFP-tagged C1 domain, PKC, or PH domain to the plasma membrane is difficult.

L15 ANSWER 23 OF 196 CA COPYRIGHT 2006 ACS on STN
AN 131:99074 CA
TI Analysis of Src **Kinase** and **Protein Kinase** C Activity by Capillary Electrophoresis and Laser-Induced **Fluorescence**
AU Wu, W. -S.; Tsai, J. -L.
CS Poison Control and Analysis Center, and Graduate Institute of Occupational Safety and Health, Kaohsiung Medical College, Kaohsiung, 80708, Taiwan
SO Analytical Biochemistry (**1999**), 269(2), 423-425
AB A sensitive capillary electrophoresis (CE)-based assay for **protein kinases** using **peptide substrates** labeled with the amino-reactive **fluorescent** cyanine dye, Cy5, was developed. Sensitivity was improved using a laser-induced **fluorescence** (LIF) detector. The assay was applied for the anal. of **protein kinase** C and Src **kinase**.

L15 ANSWER 46 OF 196 CA COPYRIGHT 2006 ACS on STN
AN 126:327015 CA
TI Conformation of the N-terminal segment of a monocysteine mutant of

troponin I from cardiac muscle

AU Dong, Wen-Ji; Chandra, Murali; Xing, Jun; Solaro, R. John; Cheung, Herbert C.

CS Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL, 35294-2041, USA

SO Biochemistry (1997), 36(22), 6745-6753

AB A mono-Cys mutant of cardiac muscle troponin I, cTnI(S5C/C81I/C98S), was generated from a mouse cTnI cDNA clone and expressed in a bacterial system. Cys-5 was modified with the **fluorescent** SH group reagent, IAANS, to probe the conformation of the N-terminal extension of the mutant and the mutant complexed with cardiac muscle troponin C. The emphasis was on the effect of **phosphorylation** of Ser-23 and Ser-24 by **protein kinase** A on the conformation of the N-terminal segment. Phosphorylation resulted in an 8-nm red shift of the emission spectrum of the attached IAANS probe and a redn. of its quantum yield by a factor of 4-5. The intensity decay of nonphosphorylated IAANS-**labeled** mutant was **complex** and had to be described by a sum of 3 exponential terms, with lifetimes in the range 0.1-5 ns. A 4th component in the range of 7-9 ns was required to describe the intensity decay of the phosphorylated mutant. Phosphorylation also reduced the weighted mean lifetime, consistent with the changes obsd. in the steady-state **fluorescence** parameters and a 33% decrease in the global rotational correlation time calcd. from anisotropy decay data. This change in correlation time suggested a decrease in the axial ratio of the protein. The **fluorescence** changes of the labeled mutant induced by phosphorylation were carried over to its complex with troponin C. The Stern-Volmer plots of acrylamide **quenching** of the steady-state **fluorescence** were essentially linear for nonphosphorylated mutant, but displayed pronounced concave downward curvatures for the **phosphorylated protein** under all conditions studied. The present results were interpreted in terms of a more compact hydrodynamic shape of the phosphorylated cTnI mutant and were consistent with a folded conformation of the N-terminal extension induced by phosphorylation of the 2 Ser residues. These conformational changes may play a role in the modulation of cardiac muscle contractility by troponin I phosphorylation.

L15 ANSWER 75 OF 196 BIOSIS on STN

AN 1995:81915 BIOSIS

TI Detection of neutral endopeptidase (NEP, enkephalinase, EC 3.4.24.11) in relation to dopaminergic and gonadoliberinergic nerve endings in the median eminence of the male rat: A **double labeling** ultrastructural study.

AU Vandenbulcke, Franck; Ciofi, Philippe; Beauvillain, Jean Claude [Reprint author]

CS INSERM U. 156, 1 place de Verdun, 59045 Lille Cedex, France

SO Journal of Neuroendocrinology, (**1994**) Vol. 6, No. 6, pp. 655-664.

AB The existence of neutral endopeptidase (Enkephalinase, NEP, E.C.3.4.24.11) in membranes of nerve endings in the rat median eminence suggests that some neuropeptides have paracrine and/or autocrine actions in this region. In vitro, neutral endopeptidase is capable of hydrolysing a variety of regulatory peptides but in vivo, many works indicate that in the central nervous system this enzyme is highly

implicated in the biological inactivation of enkephalins and tachykinins. In addition there is evidence that NEP is also involved in the inactivation of neurotensin in vivo. The modulation of the release of **gonadotrophin** releasing hormone (GnRH) is one of the documented actions of enkephalins within the median eminence. However, it is at present unclear whether enkephalins act on dopamine endings, on GnRH endings or on both. As the technical parameters and particularly the tissue fixation used to detect neutral endopeptidase are compatible with immunocytochemical detection of GnRH and tyrosine-hydroxylase (the rate limiting enzyme in the synthesis of catecholamines), two double immunolabelings were realized at the ultrastructural level to determine if GnRH and dopamine nerve endings have the enzyme inserted within their plasma membrane. Our study shows the presence of neutral endopeptidase on tyrosine-hydroxylase-immunoreactive nerve endings while presence of the enzyme on GnRH-immunoreactive nerve endings is not demonstrated. Consequently, our results provide morphological arguments for possibilities of paracrine and/or autocrine actions by neuropeptides inactivated by neutral endopeptidase on tuberoinfundibular dopaminergic nerve endings. Conversely, action of the same peptides on GnRH boutons seems more unlikely.

L15 ANSWER 168 OF 196 CA COPYRIGHT 2006 ACS on STN
AN 99:154314 CA
TI Interaction of creatine **kinase** from monkey brain with **substrate**: analysis of kinetics and **fluorescence** polarization
AU Grossman, Steven H.
CS Dep. Chem., Univ. South Florida, Tampa, FL, 33620, USA
SO Journal of Neurochemistry (1983), 41(3), 729-36
AB Titrimetric detn. of the dissocn. consts. for the binding of **substrates** to creatine **kinase** (I) from monkey brain reveals 13- and 4-fold synergism in the forward and reverse directions, resp. This synergism is expressed as a decrease in the KD for a given substrate in the ternary complex compared with the binary complex, and may be a reflection of substrate-induced conformational change. I **labeled** with **2** mols. of 5'-iodoacetamidofluorescein displays a blue shift and a decrease in **fluorescence** intensity on binding of MgADP, indicative of movement of the dye into a more hydrophobic environment and **quenching** of the extrinsic **fluorescence**. Rotational relaxation times detd. from anal. of **fluorescence** polarization of dansylated brain I decrease from 212 to 189 ns on MgADP binding. Dansylated I in 0.5% SDS has a rotational relaxation time of 135 ns. The rotational relaxation time of dansylated muscle-type isoenzyme is unaffected by MgADP and has the same value as the brain isoenzyme-MgADP complex. Polarization values at 25° for muscle and brain enzyme labeled with 3-(4-maleimidylphenyl)-7-diethylamino-4-methylcoumarin compared with limiting polarization and polarization of the free dye suggest that dye rotation is severely restricted in the muscle form, but that the dye possesses freedom of rotation in the brain form. These results suggest that, compared with the muscle isoenzyme, the brain isoenzyme is more open at the active site and more flexible overall. Binding of MgADP by brain I produces a protein more compact across 1 or both of its rotational axes, thus resembling the conformation of the muscle isoenzyme. I in brain, unlike that from muscle, is probably subject to kinetic regulation accompanied

by conformational modification, suggesting that the neurobiochem. role of the brain isoenzyme is distinct from the metabolic function of the muscle isoenzyme.

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